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Receptor Binding Studies of Soft Anticholinergic Agents

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Abbreviations:
[^{3}H]NMS: - N-[^{3}H]-methyl-scopolamine
AQC - (α-cyclopentylphenyl) 3-acetoxyquinuclidium chloride
DMPC - (α -cyclopentylphenyl) methyl-1,2-dimethylpyrrolidinium chloride
MDP - (hydroxymethyl)-3-diisopropylmethylammonium chloride-9-methylxanthene-9-carboxylate
MPC - (α -cyclopentylphenyl) 1-methylpyrrolidinium chloride
PCDT - methoxycarbonylphenylcyclopentylacetoxy-N,N-dimethyl-3-pyrrolidinium methyl sulfate
PCHA.Et - 2-phenyl-2-cyclohexen-1-carboxyl-Nα -ethoxycarbonylmethyltropinium methyl sulfate
PCHA.Me - 2-phenyl -2-cyclohexen-1-carboxyl -Nα -methoxycarbonylmethyltropinium methyl sulfate
PCHB.Et - 2-phenyl-2-cyclohexen-1-carboxyly-Nβ-ethoxycarbonylmethyltropinium bromide
PCHB.Me - 2-phenyl-2-cyclohexen-1-carboxyl-Nβ-methoxycarbonylmethyltropinium bromide
PCMS-1 - ethoxycarbonylphenylcyclopentylacetyl-*N,N* -dimethyltropinium methyl sulfate
PCMS-2 - methoxycarbonylphenylcyclopentylacetyl-*N,N* -dimethyltropinium methyl sulfate
PCPA.Et - phenylcyclopentyl-Nα -ethoxycarbonylmethyltropium methyl sulfate
PCPA.Me - phenylcyclopentyl-Nα -methoxycarbonylmethyltropium methyl sulfate
PCPB.Et - phenylcyclopentyl-Nβ-ethoxycarbonylmethyltropinum bromide
PCPB.Me - phenylcyclopentyl-Nβ-methoxycarbonylmethyltropinum bromide
PCTM - methoxycarbonylphenylcyclopentylacetoxy-ethyl-N,N,N-trimethylammonium methylsulfate
p -F-HHSiD - *p* -fluoro-hexahydro-sila-difenidol hydrochloride
PMTR - phenylmalonic atropine analogues
PMTR.MeSOMe sulfonyl tropyl 3-phenylmalonate methyl chloride salt
PMTR.TR - di-(8-methyl-8-azabicyclo[3.2.1] oct-3-yl) phenylmalonate dimethiodide
PSTR.TR - di-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl) phenylsuccinate dimethiodide
QSAR - quantitative structure-activity relationship
TMTR.Et - ethyl tropyl 3-thienylmalonate methosulfate salt
TMTR.iPr - isopropyl tropyl 3-thienylmalonate methoiodide salt

ABSTRACT

Receptor binding studies were performed on 24 soft anticholinergic agents and 5 conventional anticholinergic agents using 4 cloned human muscarinic receptor subtypes. The measured pK_i values of the soft anticholinergic agents ranged from 6.5 to 9.5, with the majority being in the range of 7.5 to 8.5. Strong correlation was observed between the pK_is determined here and the pA_2 values measured earlier in guinea pig ileum contraction assays. The corresponding correlation coefficients (r^2) were 0.80, 0.73, 0.81, and 0.78 for pK_i(m_1), pK_i(m_2), pK_i (m_3), and pK_i(m_4), respectively. Quantitative structure-activity relationship (QSAR) studies were also performed, and good characterization could be obtained for the soft anticholinergics containing at least 1 tropine moiety in their structure. For these compounds, the potency as measured by the pK_i values was found to be related to geometric, electronic, and lipophilicity descriptors. A linear regression equation using ovality (O_e), dipole moment (D), and a calculated log octanol-water partition coefficient (QLogP) gave reasonably good descriptions ($r = 0.88$) for the pK_i(m_3) values.

KEYWORDS: drug design, soft drugs, receptor binding, metabolism, drug evaluation, muscarinic antagonists

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INTRODUCTION

Muscarinic receptor antagonists, belladonna alkaloids in particular, have been used for a long time to treat a variety of clinical conditions, such as peptic ulcer, asthma, and Parkinson's disease (1). Muscarinic receptor antagonists have also been used as mydriatic/cycloplegic agents (2-4) and as experimental antiperspirants (5-7). Because of the broad range of anticholinergic effects, treatment with belladonna alkaloids directed to a certain organ system almost always induces undesirable effects in other organ systems (4). These side effects include dry mouth, blurred vision, increased heart rate, bronchodilation, reduced bronchiolar secretions, decreased gastrointestinal motility, and reduced thermoregulatory sweating (4). Even topical applications of anticholinergics can lead to unwanted systemic side effects because of their absorption and drainage into the systemic circulation. In a series of attempts to separate desired therapeutic effects from toxic effects—that is, to improve the therapeutic index—several series of novel anticholinergic agents have been designed, synthesized, and tested in our laboratories since the early 1980s based on soft drug design approaches (8-10).

Soft drugs are defined as biologically active, therapeutically useful chemical compounds (drugs) characterized by a predictable and controllable in vivo destruction (metabolism) to nontoxic moieties after achieving their therapeutic role (11,12). Soft anticholinergics were intended for topical application, for example, to be used as

antiperspirants (13) or as mydriatic agents (14,15). By incorporating an adequate metabolically labile moiety into their structure, they can be potent and locally active as anticholinergic agents, but with only minimal systemic anticholinergic effects due to their rapid metabolism in the systemic circulation. Thus, the overall therapeutic index is greatly improved (9,10).

In parallel with our efforts directed toward the development of safer anticholinergics by soft drug approaches, considerable research has been directed toward the delineation of muscarinic receptors and receptor subtypes in the past 20 years. This was done in the hope that by understanding the function of muscarinic receptors and receptor subtypes, muscarinic antagonists selectively targeted to the particular symptom(s) can be made, and, therefore, the therapeutic index can be improved (16). Three muscarinic receptor subtypes were located and characterized by biochemical and functional studies. The M_1 receptor, which is involved in behavioral and cognitive functions, exists predominantly in the brain (17,18). The heart is 1 of the rare tissues where only 1 muscarinic receptor subtype, M_2, is present (16,19). In secretory glands, the muscarinic receptors mediating the enhancement of secretion are of the M_3 subtype. M_3 also occurs in the smooth muscles of airways, the gastrointestinal tract, and the urinary bladder (20,21). With advances in molecular pharmacology in the past 15 years, 5 muscarinic receptor subtypes (m_1, m_2, m_3, m_4, and m_5) were cloned from human tissue (22-24). By functional studies, the cloned receptors have proved to be well correlated with the previously established M_1, M_2, and M_3 receptor subtypes (25,26). A tissue counterpart of m_4 has been found in the peripheral lung strip of the rabbit (27,28); however, the physiological function of M_4 has not been elucidated yet. The tissue and functional counterparts of m_5 have not been discovered yet.

Utilization of readily available cloned muscarinic receptor subtypes offers the possibility of studying the binding characteristics of muscarinic ligands in detail (25,29) and, therefore, can facilitate drug discovery efforts in the search for safer anticholinergic agents. Cloned receptors have been used to determine the potency of novel compounds and to determine the subtype selectivity of antimuscarinic agents (30-32). Currently, several muscarinic receptor subtype-selective agents are in advanced clinical trials (33,34).

The aims of the present study were (1) to establish and validate the method of receptor binding using cloned human muscarinic receptors as a tool for the discovery of soft anticholinergics; (2) to examine the potency and subtype selectivity of the existing and newly synthesized anticholinergics; and (3) to investigate the quantitative structure-activity relationship (QSAR) of these soft anticholinergic agents.

MATERIALS AND METHODS

Cloned m1, m2, m3, and m4 receptors were ordered from RBI (Boston, MA). Dissociation constants (K_D, nM) for N-[^{3}H]-methyl-scopolamine ([^{3}H]NMS) were also provided by RBI (m_1 0.166, m_2 0.24, m_3 0.11, m_4 0.06).

Pirenzipine and (±)-*p*-fluoro-hexahydro-sila-difenidol hydrochloride (*p*-F-HHSiD) were obtained from Sigma (St Louis, MO). [^{3}H]NMS was obtained from DuPont NEN Research (Boston, MA). Scintiverse BD was obtained from Fisher Scientific (Pittsburgh, PA). Atropine, scopolamine, propantheline, and all other reagents were from Sigma Chemicals (St Louis, MO). All soft anticholinergics were synthesized at the Center for Drug Discovery, University of Florida.

Synthesis

Ethyl tropyl-3-thienylmalonate methosulfate salt (4a) and isopropyl tropyl-3-thienylmalonate methoiodide salt (4b) (Figure 1).

The synthesis of **4a** and **4b** were performed by appropriate modifications of previously reported methods (35). Physicochemical data are listed below.

Ethyl 3-thienylmalonic acid (2a)

m.p. 74~75°C; ^{1}H-NMR ($CDCl_3$) δ 8.70 (brds, 1H, CO_2 **H**), 7.40-7.16 (m, 3H, C4**H**$_3$ S), 4.81 (s, 1H, C**H** (CO_2 R)$_2$), 4.80 (q, 2H-f, OC**H**$_2$ CH_3), 1.29 (t, 3H, CH_2 C**H**$_3$). Anal. for C_9 H_{10} O_4 S; Calcd.: C% 50.46, H% 4.70, S% 14.97; Found: C% 50.38, H% 4.75, S% 15.07.

Isopropyl 3-thienylmalonic acid (2b)

m.p. 82-83°C, ^{1}H-NMR ($CDCl_3$) δ 8.60 (brds, 1H, CO_2 **H**), 7.39-7.16 (m, 3H, C_4 H_3 S), 5.09 (m, 1H, C**H** (CH_3)$_2$), 4.76 (s, 1H, **H** C(CO_2 R)$_2$), 1.28 (d, 3H, CH_3), 1.24 (d, 3H, CH_3). Anal. for C_{10} H_{12} O_4 S; Calcd.: C% 52.62; H% 5.30, S% 14.05; Found: C% 52.33, H% 5.28, S% 14.27.

Ethyl tropyl 3-thienylmalonate oxalate salt (3a)

m.p. 112-114°C; ^{1}H NMR (CD_3 OD) δ 7.40- 7.18 (n, 3H,C_4 **H**$_3$ S), 5.10 (brds, 1H, H-3 of tropine), 4.95 (s, 1H, C**H** (CO_2 R)$_2$), 4.12 (q, 2H, OC**H**$_2$ CH_3), 3.80 (brds, 2H, H-1, H-5 of tropine), 2.75 (s, 3H, NC**H**$_3$), 2.50-1.90 (m, 8H, tropine), 1.25(t, 3H, CH_2 C**H**$_3$). Anal. for C_{19} H_{25} NO_8 S; Calcd. C% 53.38, H% 5.89, N% 3.28, S% 7.50; Found: C% 53.30, H% 5.94, N% 3.24, S% 7.50.

Isopropyl tropyl 3-thienylmalonate oxalate salt (3b)

m.p. 140-142°C; ^{1}H-NMR (CD_3 OD) δ 7.50-7.18 (n, 3H, C_4 **H**$_3$ S), 5.10 (brds, 1H, H-3 of tropine), 5.05 (m, 1H, C**H** (CH_3)$_2$), 4.97 (s, 1H, C**H** (CO_2 R)$_2$), 3.80 (brds, 2H, H-1 and H-5 of tropine), 2.75 (s, 3H, NC**H**$_3$), 2.50-1.95 (m, 8H, tropine), 1.30 (d, 3H, C**H**$_3$), 1.25(d, 3H, C**H**$_3$). Anal. for C_{20} H_{27} NO_8 S; Calcd. C% 54.44, H% 6.16, N% 3.17, S% 7.26; Found: C% 54.32, H% 6. 32, N% 3.07, S% 7.14.

Ethyl tropyl 3-thienylmalonate methosulfate salt (4a)

m.p. 122-124°C; ^{1}H-NMR (CO_3 OD) δ 7.40-7.10 (m, 3H, C_4 **H**$_3$ S), 5.10 (m, 1H, H-3 of tropine), 4.95 (brds, **1H** , C**H** (CO_2 R)$_2$), 4.10 (q, 2H, OC**H**$_2$ CH_3), 3.75(m, 2H, H-1 and H-5 of tropine), 3.55 (S, 3H, OC**H**$_3$), 3.10, 3.00 (s, 6H, N(CH_3)$_2$), 2.60-1.80 (n, 8H, tropine), 1.15 (t, 3H, CH_2 CH_3). Anal, for C_{19} H_{29} NO_8 S_2 Calcd. C% 49.23, H% 6.31, N% 3.02. S% 13.83; Found: C% 49.50, H% 6.31, N% 3.07, S% 13.76.

Isopropyl tropyl 3-thienylmalonate methoiodide salt (4b)

m.p. 228°C (dec): ^{1}H-NMR (CO_3 OD) δ 7.50-7.10 (m, 3H, C_4H_3S), 5.15 (m, 1H, H-3 tropine), 5.05 (m, 1H, C**H** ($CH_3)_2$), 4.80 (brds, 1H, C**H** (CO_2 R)), 3.85(m, 2H, H-1 and H-5 of tropine), 3.18, 3.10 (s, 6H, N($CH_3)_2$), 2.70-1.90 (n, 8H, tropine), 1.25, 1.20 (d, 6H, CH(C**H**$_3)_2$). Anal. for $C_{19}H_{28}INO_4S$; Calcd. C% 46.25, H% 5.72, N% 2.84, S% 6.50; Found: C% 46.17, H%, 5.70, N% 2.83, S% 6.46.

Synthesis of di-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)phenylmalonate dimethiodide (10a) and di-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)phenylsuccinate dimethiodide. (ditropinyl phenylsuccinate ester diquaternary, 10b).

The synthesis of **10a** and **10b** were performed by appropriate modifications of previously reported methods(35). The physicochemical data are listed below.

Di-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)phenylmalonate dimethiodide (10a).

FAB-MS (w/NBA + Na) 441.2 (M-CH_3), 583.2 (M + I), 733.1 (M + 21 + Na). ^{1}H-NMR ($CDCl_3$) δ 7.5 (s, 5H, C_6H_5); 5.2 (t, 2H, 3-tropyl Hs); 4.76 (s, 1H, C_6H_5-C**H**); 3.1 and 3.18 (two singlets, 12 H, two N$^+$ ($CH_3)_2$), 3.8 (broad doublet, 4H) and 2.8-1.65 (m, 16 H) the rest of tropyl hydrogens.

Di-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)phenylsuccinate dimethiodide (ditropinyl phenylsuccinate ester diquaternary, 10b).

FAB-MS (w/Gly) 455.2 (M-CH_3). ^{1}H-NMR (DMSO-d_6) d 7.38 (s, 5H, C_6H_5); 5.0 (m, 2H, 3-tropyl Hs); 4.2 (m, H, C_6H_5-C**H**); 3.18, 3.15, 3.6, and 3.0 four singlets each for 3H (2 N($CH_3)_2$), 3.9-1.2 (multiplets, 20H, rest of tropine Hs).

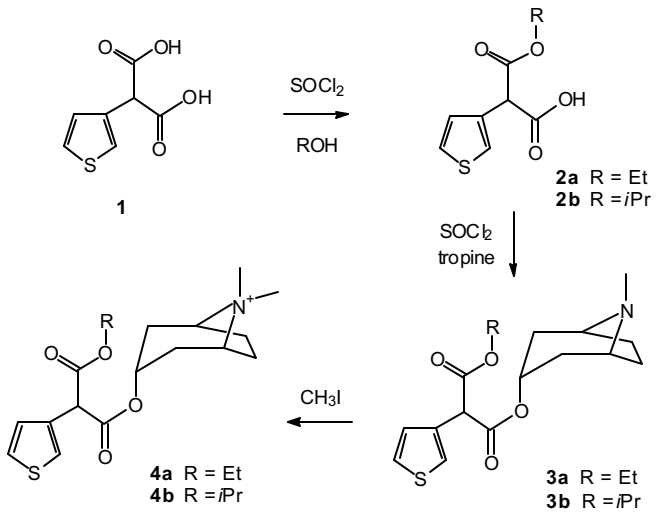


Figure 1. Synthesis of ethyl tropyl 3-thienylmalonate methosulfate salt (TMTR.Et, **4a**) and isopropyl tropyl 3-thienylmalonate methoiodide salt (TMTR.iPr, **4b**).

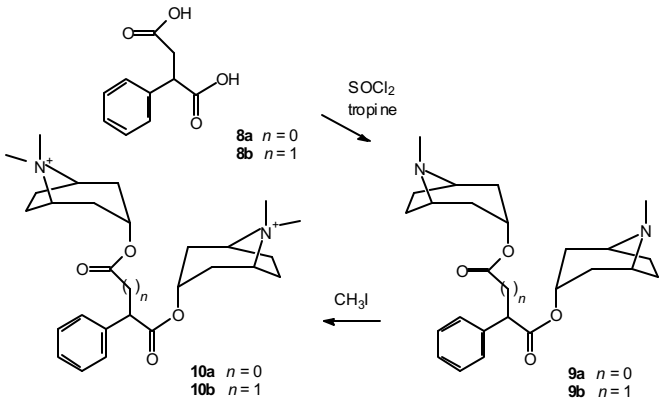


Figure 2. Synthesis of di-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl) phenylmalonate dimethiodide (PMTR.TR, **10a**) and di-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl) phenylsuccinate dimethiodide (PSTR.TR, **10b**).

Stability Studies of Soft Anticholinergics in the Receptor Environment

On the basis of previous studies (9,10), the most labile soft anticholinergics from each series of soft anticholinergics were chosen to test the integrity of the compound during incubation with the esterase enzyme inhibitor. AQC, PMTR.Et, PCMS-1, PCDT, and PCTM were chosen to test the stability in the receptor media.

Method

The incubation buffer was phosphate-buffered saline (PBS), with 10 mM NaF as esterase enzyme inhibitor. The incubation mixture (1 mL) contained 500 µL diluted receptor membrane (prepared in incubation buffer), 100 µL of 100 mM soft anticholinergic (dissolved in buffer), and 400 µL buffer. Incubation was carried out at room temperature (about 23°C) for 60 minutes. At the end of incubation, 0.1 mL of aliquots were removed, and 0.9 mL 5% DMSO in acetonitrile was added, vortexed, and centrifuged at 1000 rpm for 15 minutes. Aliquots of the supernatant (20 µL) were injected into HPLC for analysis. The HPLC system was adopted from previously reported studies (36).

Radioligand Binding Assay

Binding studies were performed with [^{3}H]NMS following the protocol from RBI. The binding buffer, pH 7.4, consisted of 0.15 M NaCl, 1.5 mM KH_2PO_4, and 2.7 mM Na_2HPO_4. NaF 10 mM was added to the buffer as an esterase inhibitor. The assay mixture (1 mL) contained 100 µL diluted membranes (receptor proteins, final concentration: m_1 25 µg/mL, m_2 42 µg/mL, m_3 15.9 µg/mL, m_4 20 µg/mL). Final concentrations of [^{3}H]NMS for the m_2-m_4 binding studies were 0.5 nM and 1 nM for m_1. Specific binding was defined as the difference between the [^{3}H]NMS binding in the absence and presence of 1 µM atropine. Incubation was carried out at room temperature for 60 minutes. The assay was terminated by filtration through a Whatman GF/B filter (presoaked with 0.5% polyethyleneimine). The filter was then washed 3 times with 10 mL ice-cold binding buffer,

Table 1. Binding parameters of reference compounds at 4 muscarinic receptor subtypes. The affinity estimates were derived from [^{3}H]NMS displacement experiments and represent the mean (±SEM, n = 3-5) for the negative logarithm of K_i; Hill coefficients are given in parentheses.

	Subtypes of Cloned Muscarinic Receptors				pA_2
	m_1	m_2	m_3	m_4	
Atropine	**9.34 ± 0.04**	**8.95 ± 0.02**	**9.15 ± 0.11**	**8.94 ± 0.13**	**8.50[a]**
	(0.94 ± 0.05)	**(0.97 ± 0.01)**	**(1.10 ± 0.03)**	**(1.02 ± 0.05)**	
Scopolamine	**8.95 ± 0.31**	**8.68 ± 0.08)**	**9.41 ± 0.07**	**9.47 ± 0.06**	**9.50[b]**
	(1.00 ± 0.04)	**(1.02 ± 0.05)**	**(0.97 ± 0.11)**	**(1.11 ± 0.04)**	
Pirenzepine	**8.29 ± 0.12**	**6.0 ± 0.12**	**6.46 ± 0.07**	**7.64 ± 0.05**	**N/A[c]**
	(0.96 ± 0.03)	**(0.92 ± 0.03)**	**(0.94 ± 0.11)**	**(0.94 ± 0.08)**	
***p*-F-HHSiD**	**7.76 ± 0.03**	**6.63 ± 0.04**	**7.86 ± 0.05**	**7.51 ± 0.01**	**N/A[c]**
	(0.97 ± 0.03)	**(1.03 ± 0.05)**	**(1.07 ± 0.08)**	**(0.91 ± 0.04)**	
Propantheline	**9.66 ± 0.12**	**9.48 ± 0.22**	**10.04 ± 0.14**	**10.24 ± 0.11**	**8.93[b]**
	(0.94 ± 0.05)	**(0.94 ± 0.03)**	**(1.07 ± 0.04)**	**(1.09 ± 0.03)**	

[a] Data from Bodor et al, 1980 (13).
[b]Data from Kumar and Bodor, 1996 (10).
[c]N/A: not available.

transferred to vials, and added with 10 mL of Scintiverse liquid. Finally, detection was performed on a Packard 31800 liquid scintillation analyzer (Packard Instrument, Downer Grove, IL).

Data Analysis

To obtain the Hill coefficients, n, data from the binding experiment were fitted to the following equation: % [^{3}H]NMS bound = $100 - [100x^n / k / (1 + x^n/k)]$. Next, they were fitted to the % [^{3}H]NMS bound = $100 - [100x^n / IC_{50} / (1+x^n/IC_{50})]$ equation in order to obtain the IC_{50} values. Here, x denotes the concentration of the tested compound (in a series concentration). K_i was derived by the method of Cheng and Prusoff (37): $K_i = IC_{50}/(1 + L/K_D)$, where L is the concentration of the radioligand, IC_{50} is the concentration of drug causing 50% inhibition of specific radioligand binding, and K_D is the dissociation constant of the radioligand-receptor complex. Data were analyzed by a nonlinear least-squares curve-fitting procedure using the Scientist software (MicroMath, Salt Lake City, UT).

Guinea pig ileum assay (pA_2 value)

Standard guinea pig ileum method (35,38) was used to determine the pA_2 values of the soft drugs. Dose response curves were plotted, and pA_2 values were calculated using a Schild plot.

Quantitative structure-activity relationship (QSAR)

All structures were optimized using the AM1 advanced semi-empirical quantum chemical method (39) on a Silicon Graphics Origin 2000 server with the Sybyl molecular modeling program (Tripos, St Louis, MO). Keywords used were as follows: AM1 PRECISE POLAR CHARGE = 1. Twenty-five descriptors were studied in our search for possible relevant parameters: molecular weight (MW); van der Waals molecular volume (V) and surface area (S) together with the corresponding ovality (O) as a shape descriptor (40); effective van der Waals molecular volume (V_e) and surface area (S_e) together with the corresponding ovality (O_e) calculated with a new procedure and a slightly different van der Waals radii set (41); AM1 calculated dipole moment (D), average polarizability (α), ionization energy (I), and heat of formation; HOMO-LUMO energies (E_{HOMO}, E_{LUMO}); absolute electronegativity (χ), calculated as the negative of the average HOMO and LUMO energies, and absolute hardness (η), calculated as half the HOMO-LUMO difference; calculated log octanol-water partition coefficient (log $P_{o/w}$) using BLOGP (40), QLogP (41,42), and MLOGP (43); calculated log water solubility (log W) using BLOGW (44); AM1-calculated partial atomic charges on the quaternary nitrogen (q_N) atom and the sp^2 carbon and oxygen atoms of the ester moiety ($q_{C=}$, $q_{O=}$); the distance between the quaternary nitrogen atom and the sp^2 carbon of the ester moiety (d_{C-N}); and the inaccessible solid angle around these atoms (Ω_C, Ω_N) as a measure of steric hindrance (45).

RESULTS

Stability studies showed that all tested compounds retained at least 90% integrity during the 60-minute incubation period. Table 1 presents the mean pK_i ± SEM values obtained for each compound in the receptor binding studies. The pK_i values obtained by us for atropine, scopolamine, *p*-F-HHSiD (m_3 selective agent), and pirenzepine (m_1 selective agent) were in good agreement with published data (25,26,29,31). The Hill coefficients, n, for the above compounds were not significantly

different from unity, indicating that drug-receptor interactions obeyed the law of action and that binding took place at only 1 site. This further validates the method used here to evaluate the binding of the soft anticholinergics. However, the Hill coefficients obtained for some soft anticholinergics were significantly different from unity. Theoretically, n is an integer that reflects the number of molecules binding to a specific drug receptor. Normally, the binding of classical antagonists to muscarinic receptors is well described by the simple Langmuir isotherm, indicating a Hill coefficient close to unity (46). Low Hill coefficients are often attributed to either recognition by the antagonist of more than 1 receptor site or conformation, or to interaction of the antagonist with a second binding site on the receptor molecule, which causes a negative cooperative effect on the first site (30,47,48). None of these seems to apply in our situation. It is possible that small amounts of the inactive metabolite generated from the hydrolysis of soft drugs interfered with the binding of the parent compounds at the extremely low concentrations used in these studies (10^{-4} to 10^{-11} M), causing the Hill coefficients (n) to deviate from unity. In preliminary experiments, for several soft anticholinergics bound to m_3, we observed n values below 0.8 before adding the esterase inhibitor NaF. Addition of NaF boosted the Hill coefficients to values around 0.8. Nevertheless, the exact reasons as to why n was significantly different from unity still needs further investigation. It should be pointed out that the soft anticholinergics concentrations used in the receptor binding studies were much lower than those used in the stability studies because of the HPLC detection limit. Even if the Hill coefficient (n) strongly influences the B_{max} (maximum binding) value, it has very little effect on the estimation of IC_{50} if a sigmoidal E_{max} model is used (49). Therefore, pK_i values, which are derived from IC_{50} (37), should represent valid potency estimates even under the present experimental conditions.

A validation of the receptor binding measurements on cloned muscarinic receptors is the correlation of pK_i values with the pA_2 values determined by the guinea pig ileum contraction method. The correlation between pA_2 and different receptor subtype pK_i are fairly good as characterized by the r^2 values (n = 18): 0.803, 0.734, 0.813, and 0.781 for m_1, m_2, m_3, and m_4, respectively. Even if differences are relatively small, the strongest correlation was observed between pA_2 and pK_i (m_3) values (Figure 3), which is in agreement with the fact that M_3 mediates smooth muscle in airway and gastrointestinal tract contraction (34). Determination of the pA_2 value for guinea pig ileum contraction has been a classical functional study for anticholinergic affinity toward the M_3 receptor. For soft anticholinergics, the pA_2 values obtained from these studies were generally comparable to the pK_i values obtained from m_3 binding studies, even though in most cases, the pA_2 values were somewhat lower than the corresponding pK_i values of m_3 binding. Relative values for each tested compound were essentially the same for either method. Because the receptor-binding assay allows faster screening, it has an advantage over pA_2 value determination as a method to measure relative potency in anticholinergic compounds. Furthermore, the latest research indicates that muscarinic receptors in guinea pig ileum are heterogeneous with a major M_2 receptor population (~80%) and a minor M_3 population (~20%). The function of the minor M_3 population is clearly related to contraction, but the function of the predominate M_2 population is yet unclear (50). Therefore, m_3 receptor-binding data provide a more reliable estimate of intrinsic activity toward the M_3 receptor.

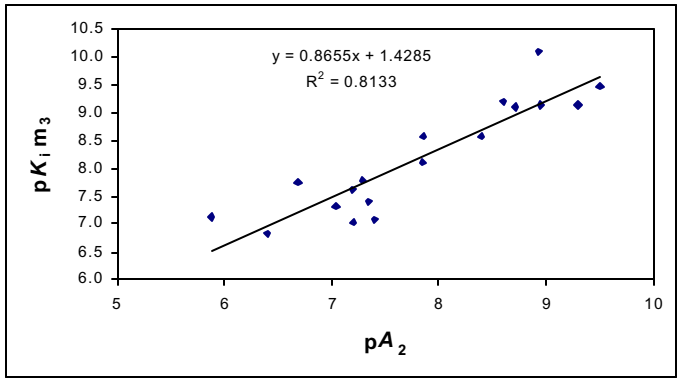


Figure 3. Correlation between pA_2 and pK_i (m_3) data for soft anticholinergics.

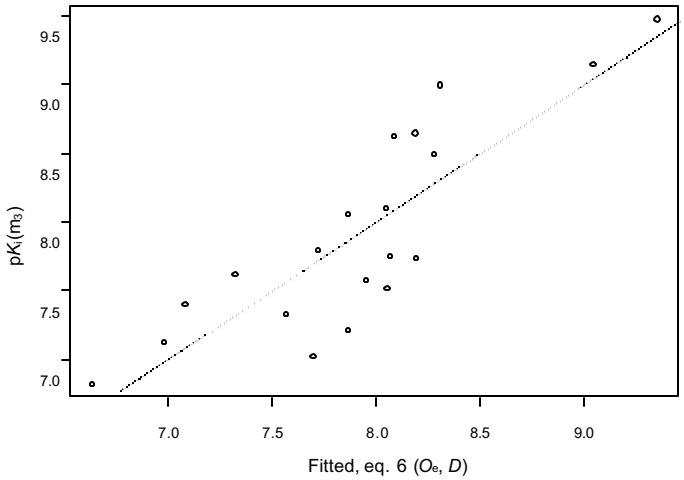


Figure 4. Correlation between experimental and fitted (eq. 6, Table 8) pK_i (m_3) values.

Twenty-four soft anticholinergic agents were included in the present study. They can be divided into 2 major groups: compounds containing at least 1 tropine moiety within their structure (**Table 2** and **Table 4**) and compounds that contain no such moiety (**Table 3** and **Table 5**). Compounds in the first group were designed by using the inactive metabolite-based soft drug approach (11,12). Those in the second group, except PCDT and PCTM, were designed by using the soft analogue approach (11,12).

Table 2. Binding parameters of soft anticholinergics at m_1, m_2, m_3, and m_4 receptors. The affinity estimates were derived from [^{3}H]NMS displacement experiments and represent the mean (±SEM, n = 3-5) for the negative logarithm of K_i; Hill coefficients are given in parentheses.

	Subtypes of Cloned Muscarinic Receptors				pA_2
	m_1	m_2	m_3	m_4	
PMTR.Et	8.32 ± 0.11	7.74 ± 0.06	8.16 ± 0.03	7.78 ± 0.02	7.85[b]
	(1.04 ± 0.02)	(0.66 ± 0.12)	(0.71 ± 0.06)	(0.97 ± 0.13)	
PMTR.cHx	8.01 ± 0.14	7.54 ± 0.04	7.39 ± 0.02	7.47 ± 0.04	7.35[b]
	(0.87 ± 0.05)	(0.66 ± 0. 12)	(1.06 ± 0.13)	(0.97 ± 0.02)	
PMTR.Hx	7.33 ± 0.04	6.91 ± 0.03	6.82 ± 0.03	6.87 ± 0.04	6.40 [b]
	(0.95 ± 0.12)	(0.84 ± 0.04)	(0.94 ± 0.03)	(0.78 ± 0.03)	
PCMS-1	7.98 ± 0.21	7.85 ± 0.14	8.18 ± 0.17	8.20 ± 0.11	7.19[c]
	(0.93 ± 0.08)	(0.94 ± 0.07)	(0.89 ± 0.11)	(0.86 ± 0.11)	
PCMS-2	7.28 ± 0.04	7.31 ± 0.04	7.32 ± 0. 12	7.42 ± 0.21	7.02[c]
	(1.02 ± 0.03)	(0.84 ± 0.14)	(0.89 ± 0.12)	(0.96 ± 0.21)	
PCPA.Me [d]	7.33 ± 0.10	7.14 ± 0.06	7.51 ± 0.15	N/A[e]	N/A
	(0.76 ± 0.03)	(0.85 ± 0.07)	(0.80 ± 0.06)		
PCPA.Et[d]	7.00 ± 0.08	6.94 ± 0.09	7.21 ± 0.2	N/A	N/A
	(0.88 ± 0.05)	(0.85 ± 0.07)	(1.01 ± 0.03)		
PCPB.Me[d]	7.65 ± 0.01	7.54 ± 0.18	7.75 ± 0.10	7. 42 ± 0.05	N/A
	(0.83 ± 0.04)	(0.78 ± 0.02)	(0.73 ± 0.01)	(0.93 ± 0.02)	
PCPB.Et[d]	7.42 ± 0.04	7.20 ± 0.03	7.57 ± 0.08	N/A	N/A
	(0.87 ± 0.02)	(0.83 ± 0.04)	(0.75 ± 0.01)		
PCHA.Me[d]	7.89 ± 0.07	7.38 ± 0.07	8.49 ± 0.02	8.11 ± 0.06	N/A
	(0.83 ± 0.05)	(1.07 ± 0.07)	(0.81 ± 0.04)	(1.07 ± 0.02)	
PCHA.Et[d]	7.98 ± 0.04	7.70 ± 0.06	8.62 ± 0.05	8.17 ± 0.03	N/A
	(0.91 ± 0.05)	(0.80 ± 0.06)	(0.87 ± 0.05)	(0.82 ± 0.05)	
PCHB.Me[d]	7.86 ± 0.03	7.73 ± 0.10	8.99 ± 0.01	8.43 ± 0.07	N/A
	(0.78 ± 0.05)	(0.91 ± 0.10)	(0.81 ± 0.02)	(0.90 ±0.02)	
PCHB.Et[d]	7.93 ± 0. 04	7.97 ± 0.03	8.64 ± 0.05	8.20 ± 0.06	N/A
	(0.86 ± 0.01)	(0.88 ± 0.03)	(0.87 ± 0.06)	(0.91 ± 0.07)	
TMTR.Et	7.57 ± 0.08	7.29 ± 0.22	7.73 ± 0.08	7.40 ± 0.01	N/A
	(0.85 ± 0.06)	(0.94 ± 0.02)	(0.68 ± 0.02)	(0.76 ± 0.03)	
TMTR.iPr	8.00 ± 0.05	7.82 ± 0.13	8.05 ± 0.05	8. 18 ± 0.21	N/A
	(0.96 ± 0.01)	(0.85 ± 0.34)	(0.68 ± 0.04)	(0.79 ± 0.05)	
PMTR.TR	7.18 ± 0.02	7.79 ± 0. 02	7.79 ± 0. 20	7.72 ± 0.10	7.29
	(0.57 ± 0.04)	(0.65 ± 0.10)	(0.71 ± 0.01)	(0.78 ± 0.21)	
PSTR.TR	6.50 ± 0.13	6.76 ± 0.03	7.12 ± 0.05	N/A	5.88
	(0.56 ± .21)	(0.58 ± 0.04)	(0.50 ± 0.03)		
PMTR.MeSOMe	7.25 ± 0.04	6.90 ± 0.10	7. 02 ± 0.09	6.91 ± 0.18	7.20
	(0.59 ± 0.01)	(0.59 ± 0.01)	(0.67 ± 0.01)	(0.96 ± 0.01)	

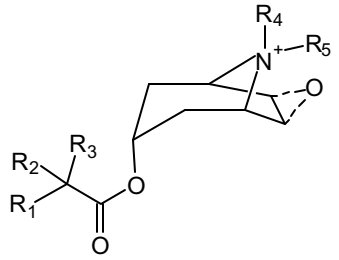
[a] Data from Bodor et al, 1980 (13).
[b] Data from Kumar and Bodor, 1996 (10).
[c] Receptor binding and pA_2 data from Juhász et al, 1998 (9).
[d] Receptor binding data adapted from Huang, 1999 (60).
[e] N/A: not available.

Table 3. Binding parameters of soft anticholinergics at m_1, m_2, m_3, and m_4 receptors. The affinity estimates were derived from [^{3}H]NMS displacement experiments and represent the mean (±SEM, n = 3-5) for the negative logarithm of K_i; Hill coefficients are given in parentheses.

	Subtypes of Cloned Muscarinic Receptors				pA_2
	m_1	m_2	m_3	m_4	
DMPC	**9.25 ± 0.03**	**8.85 ± 0.04**	**9.14 ± 0.06**	**9.42 ± 0.02**	**9.3[a]**
	(0.94 ± 0.08)	**(1.13 ± 0.04)**	**(0.76 ± 0.21)**	**(0.93 ± 0.05)**	
MPC	**8.48 ± 0.11**	**8.34 ± 0.02**	**8.56 ± 0.11**	**8.7 ± 0.01**	**8.4[a]**
	(0. 86 ± 0.21)	**(0.94 ± 0. 21)**	**(0.97 ± 0.18)**	**(1.05 ± 0.01)**	
AQC	**9.05 ± 0.23**	**9.11 ± 0.03**	**9.20 ± 0.12**	**8.85 ± 0.03**	**8.55[a]**
	(1.01 ± 0.03)	**(0.84 ± 0.13)**	**(1.04 ± 0.05)**	**(0.96 ± 0.08)**	
MDP[c]	**8.56 ± 0.21**	**8.71 ± 0.02**	**8.58 ± 0.14**	**8.25 ± 0.02**	**7.95[a]**
	0.9 7 ± 0.03	**0.90 ± 0.11**	**0.87 ± 0.13**	**0.97 ± 0.04**	
PCTM[b]	**6.62 ± 0.12**	**6.54 ± 0.20**	**6.46 ± 0.29**	**6.84 ± 0. 21**	**6.72**
	(0.93 ± 0.03)	**(1.04 ± 0.08)**	**(0.9 ± 0.03)**	**(0.88 ± 0.14)**	
PCDT[b]	**7.54 ± 0.05**	**6.95 ± 0.02**	**7.81 ± 0.01**	**8.02 ± 0.02**	**7.37**
	(0.98 ± 0. 11)	**(1.02 ± 0.11)**	**(1.03 ± 0. 04)**	**(0.87 ± 0.06)**	

[a]Data from Bodor et al, 1980 (13).
[b]pA_2 and receptor binding data from Ji et al, 2000 (8).
[c]For structure see Brouillette et al, 1996 (36).

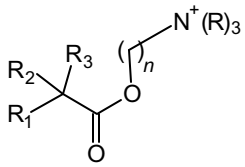
Table 4. Soft anticholinergics[a] with a tropine moiety in their structure.



Compound	R_1	R_2	R_3	R_4	R_5	Sc.O[b]
methatropine	**Ph**	**H**	**CH_2OH**	**CH_3**	**CH_3**	**-**
methscopolamine	**Ph**	**H**	**CH_2OH**	**CH_3**	**CH_3**	**+**
PMTR.Et	**Ph**	**H**	**COO-ethyl**	**CH_3**	**CH_3**	**-**
PMTR.cHx	**Ph**	**H**	**COO-cyclohexyl**	**CH_3**	**CH_3**	**-**
PMTR.Hx	**Ph**	**H**	**COO-*n*-hexyl**	**CH_3**	**CH_3**	**-**
PCMS-1	**Ph**	**Cyclopentyl**	**COO-ethyl**	**CH_3**	**CH_3**	**-**
PCMS-2	**Ph**	**Cyclopentyl**	**COO-methyl**	**CH_3**	**CH_3**	**-**
PCPA.Et	**Ph**	**Cyclopentyl**	**H**	**CH_3**	**COO-ethyl**	**-**
PCPA.Me	**Ph**	**Cyclopentyl**	**H**	**CH_3**	**COO-methyl**	**-**
PCPB.Et	**Ph**	**Cyclopentyl**	**H**	**COO-ethyl**	**CH_3**	**-**
PCPB.Me	**Ph**	**Cyclopentyl**	**H**	**COO-methyl**	**CH_3**	**-**
PCHA.Et	**H**	**2-phenyl-2-cyclohexenen**		**CH_3**	**COO-ethyl**	**-**
PCHA.Me	**H**	**2-phenyl-2-cyclohexenen**		**CH_3**	**COO-methyl**	**-**
PCHB.Et	**H**	**2-phenyl-2-cyclohexenen**		**COO-ethyl**	**CH_3**	**-**
PCHB.Me	**H**	**2-phenyl-2-cyclohexenen**		**COO-methyl**	**CH_3**	**-**
TMTR.Et	**H**	**Thienyl**	**COO-ethyl**	**CH_3**	**CH_3**	**-**
TMTR.iPr	**H**	**Thienyl**	**COO-*i*-propyl**	**CH_3**	**CH_3**	**-**
PMTR.MeSOMe	**Ph**	**H**	**$-COOCH_2SOCH_3$**	**CH_3**	**CH_3**	**-**

[a]For comparison, methatropine and methscopolamine were also included.
[b]Indicates the presence or absence of the scopolamine oxygen.

Table 5. Soft anticholinergics with no tropine moiety in their structure.



Compound	R_1	R_2	R_3	$-N^+(R)_3$	*n*
DMPC	**Ph**	**Cyclopentyl**	**H**	**1,2-Dimethylpyrrolidinium**	**1**
MPC	**Ph**	**Cyclopentyl**	**H**	**N-Methylpyrrolidinium**	**1**
AQC	**Ph**	**Cyclopentyl**	**H**	**3-Acetoxyquinuclidium**	**1**
PCDT	**Ph**	**Cyclopentyl**	**$COOCH_3$**	**N,N-Dimethyl-3-pyrrolidinium[a]**	**2[a]**
PCTM	**Ph**	**Cyclopentyl**	**$COOCH_3$**	**N,N,N-trimethylammonium**	**2**

[a]Carbon #3 of the pyrrolidine moiety connects to the oxygen atom.

Table 6. Reference compounds used to obtain predicted log distribution coefficient (log D) with the QLogP method.

Compound	*Formula*	*log* D[a]	***Rm***[b]	V_e	N	*QLogP*
tetramethyl ammonium (iodide)	**$C_4H_{12}N_1$**	**−3.92**		**76.63**	**9**	**−4.07**
acetylcholine (bromide)	**$C_7H_{16}N_1O_2$**	**−4.12**	*****	**127.20**	**11**	**−3.91**
triethyl methylammonium (iodide)	**$C_7H_{18}N_1$**	**−2.13**		**119.02**	**9**	**−2.72**
trimethyl butylammonium (iodide)	**$C_7H_{18}N_1$**	**−2.05**	**avg**	**118.93**	**9**	**−2.72**
trimethyl *s*-butylammonium (iodide)	**$C_7H_{18}N_1$**	**−1.61**		**119.03**	**9**	**−2.72**
trimethyl *t*-butylammonium (iodide)	**$C_7H_{18}N_1$**	**−2.08**		**119.05**	**9**	**−2.72**
furane, 2-trimethylammoniomethyl (iodide)	**$C_8H_{14}N_1O_1$**	**−2.40**	**√**	**119.70**	**9**	**−2.70**
tetraethyl ammonium (chloride)	**$C_8H_{20}N_1$**	**−2.59**		**133.11**	**9**	**−2.27**
tetraethyl ammonium (iodide)	**$C_8H_{20}N_1$**	**−2.82**	*****	**133.11**	**9**	**−2.27**
phenyl trimethyl ammonium (iodide)	**$C_9H_{14}N_1$**	**−2.33**	**√**	**122.31**	**9**	**−2.62**
furane, 5-methyl, 2-trimethylammoniomethyl (iodide)	**$C_9H_{16}N_1O_1$**	**−1.85**	**√**	**133.50**	**9**	**−2.26**
trimethylammonium, cyclopentyl (bromide)	**$C_9H_{20}N_1$**	**−2.00**		**138.57**	**9**	**−2.10**
trimethylammonium, cyclopentyl (iodide)	**$C_9H_{20}N_1$**	**−2.00**		**138.57**	**9**	**−2.10**
trimethyl hexylammonium (iodide)	**$C_9H_{22}N_1$**	**−1.84**	*****	**146.94**	**9**	**−1.83**
benzyl trimethyl ammonium (chloride)	**$C_{10}H_{16}N_1$**	**−2.63**	**avg**	**136.43**	**9**	**−2.17**
arecaidine, ethyl ester, N-methyl (iodide)	**$C_{10}H_{18}N_1O_2$**	**−2.35**		**154.79**	**11**	**−3.03**
trimethylammonium, (3-methyl)cyclopentyl (iodide)	**$C_{10}H_{22}N_1$**	**−1.50**	**√**	**152.71**	**9**	**−1.65**
phenyl diethylmethyl ammonium (iodide)	**$C_{11}H_{18}N_1$**	**−2.22**		**150.47**	**9**	**−1.72**
trimethyl octylammonium (iodide)	**$C_{11}H_{26}N_1$**	**−1.21**		**175.04**	**9**	**−0.94**
tripropyl ethylammonium (iodide)	**$C_{11}H_{26}N_1$**	**−2.19**		**175.17**	**9**	**−0.93**
methylatropine (nitrate)	**$C_{18}H_{26}N_1O_3$**	**−2.22**		**247.22**	**13**	**−1.47**

[a]Experimental distribution coefficients taken from reference (61).
[b]Remarks: Starred (*) and checked (√) values considered to be more reliable experimental values; "avg" denotes those values that represent an average, as more experimental data were available.

PMTR.Et, PMTR.cHx, and PMTR.Hx are soft analogues of atropine (35,51). These 3 compounds were obtained by replacing the OH groups of the methatropine moiety with different ester groups. Because atropine does not show muscarinic subtype selectivity, it is not expected that these compounds will exhibit subtype selectivity. The rank order of potency is generally $m_1 \geq m_3 >> m_2$. Studies of these 3 compounds indicated that substitution of the OH group with an ester in methatropine tends to reduce potency. This will be further discussed in the QSAR section of this paper. PMTR.MeSOMe is a similar structure containing a sulphone group, and it has moderate potency.

DMPC, MPC, AQC, and MDP (Table 3 and Table 5) were intended for the suppression of perspiration. MDP is an analogue of propantheline and, hence, a possible anti-ulcer agent as well. All of these compounds were designed using the soft analogue approach, and they displayed higher potency in receptor-binding studies ($pK_i > 8.5$) than most of the soft drugs designed using the inactive metabolite-based approach ($pK_i < 8.5$). None of these compounds showed muscarinic receptor subtype selectivity. PCTM and PCDT are analogues of glycopyrrolate. Slight subtype selectivity was observed for PCTM (m_3/m_2 binding ratio of around 8).

TMTR.Et (4a), TMTR.iPr (4b), PMTR.TR (10a), and PSTR.TR (10b) are newly synthesized soft anticholinergics. Their syntheses proceeded smoothly following appropriate modifications of previously established methodology (35) (Figure 1, Figure 2). TMTR.Et and TMTR.iPr are soft anticholinergics similar to PMTR.Et, PMTR.cHx, and PMTR.Hx, but with a thienyl moiety replacing the phenyl in an attempt to enhance potency. However, this replacement did not significantly increase the potency. PMTR.TR and PSTR.TR (Figure 2) contain 2 tropine moieties in their structures. Binding results indicated that the increase of the number of tropine moieties did not enhance the potency either.

PCPA.Me, PCPA.Et, PCPB.Me, PCPB.Et, PCHA.Me, and PCHA.Et are soft anticholinergics with the "soft spot" (the metabolically labile moiety) introduced at the quaternary nitrogen head. Of these compounds, only PCHA.Me and PCHB.Me showed muscarinic receptor subtype selectivity (m_3/m_2).

Quantitative structure-activity relationships (QSARs)

Because the compounds included in the study contain quaternary nitrogens and the QLogP method was not previously tested for estimation of the log distribution coefficient (log *D*) of such compounds, the possibility of extending this model for such predictions was evaluated. QLogP uses only 2 parameters to estimate the log octanol-water partition coefficient, $\log P_{o/w} = 0.032V_e - 0.723N$, where V_e is the calculated effective van der Waals molecular volume and *N* is a mainly additive parameter assigned by a fully automated algorithm that has integer values and is related to hydrogen bond acceptor ability (41,42). Because the distribution of charged molecules is pH- and counterion-dependent (42), experimentally determined log *D* values of permanently charged molecules are less reliable than experimental log partition coefficients (log *P*), which by definition describe the partitioning of neutral molecules. Nevertheless, as data in Table 6 indicate, the extension of QLogP could be reasonably well performed by assuming a contribution of $N = 9$ for the positively charged quaternary nitrogen functionality of these molecules. It is reassuring that this $N = 9$ value is the same as the one used earlier in the extension of the QLogP model to describe the distribution coefficient of quaternary pyridinium compounds (42), and this can be regarded as an additional proof of the consistency of this method.

Because of the structural diversity of the compounds included in the present study, general size, shape, lipophilicity, solubility, electronic, and steric parameters were employed in the present QSAR analysis. Our initial efforts to correlate activity with physicochemical parameters for all soft anticholinergics included in this study did not prove very successful: None of the included parameters was found to be a good descriptor of the measured receptor binding data (pK_i). Size descriptors (eg, *V*, *S*, MW) proved again to be the best, but they were far less relevant than previously found for pA_2 data (9). This is true even despite the relatively good correlation found between pA_2 and pK_i data (Figure 1). For example, V_e accounts for close to 65% of the variance in pA_2 ($r^2 = 0.63$, $n = 18$), but for only about 16% of the variance in m_3 pK_i ($r^2 = 0.16$, $n = 29$) (Figure 2). Note, however, that compounds with available pK_i data contain larger structural variety than those with available pA_2 data. Inclusion of second- or higher-order terms gave no significant improvement.

However, when only the soft anticholinergics with 1 or 2 tropine moiety in their structures were included, significantly improved correlation was found between activity and geometric descriptor, such as volume (V_e), surface area (S_e), and ovality (O_e). Significant correlation was also found between activity and electronic descriptor(s) such as dipole moment (*D*). It is not unreasonable to separate the soft anticholinergics with a tropine moiety in their structure from the other soft anticholinergics in the present study. In the compounds containing the tropine moiety, 3 carbon atoms separate the quaternary nitrogen head from the ester function. In contrast, in the compounds of Table 5, there is a significantly shorter separation of only 1 (or sometimes 2) carbons. It is very likely that for the corresponding 2 series of soft anticholinergics, there are 2 distinct receptor-ligand complexes in terms of conformation, since the spatial structure of muscarinic receptors was considered to contain 4 binding sites (52).

In order to establish a quantitative relationship between physicochemical parameters and activities (pK_is), the correlations between pK_i (m_1 to m_3) and the 25 physicochemical parameters included were examined with the S-Plus program (Mathsoft, Seattle, WA). Parameters showing statistically

significant correlations with the pK_i values were selected, and the intercorrelations among these parameters were further examined. Of the parameters showing strong intercorrelation ($r > 0.8$), only 1 was kept for the final study. For example, strong intercorrelation was found among O, S, V, O_e, S_e, and V_e; therefore, only O_e was kept for the final study as it showed the strongest correlation with the pK_i values. For the final QSAR study, 8 physicochemical descriptors were selected with this procedure (Table 7), including a geometric descriptor (O_e), a lipophilicity descriptor (QLogP), a water-solubility descriptor (BLOGW), and 5 electronic descriptors ($D, I, \chi, q_{C=}$, and $q_{O=}$).

Linear regressions were obtained by using the stepwise regression mode of the SPlus program. Following the usual methodology, only those parameters that improved (when added) or decreased (when deleted) the goodness-of-fit in a statistically significant manner were kept in the final regression model.

Table 8 presents the obtained regression equations for $pK_i(m_1)$, $pK_i(m_2)$, and $pK_i(m_3)$, and Table 9 gives a correlation matrix between selected physicochemical descriptors and pK_i values. QSAR for $pK_i(m_4)$ was not performed at this time because the physiological significance of m_4 has not been clearly established yet. For all 3 pK_is, O_e was a major factor. Ovality, O_e, is a parameter that describes the overall size and shape of the molecules (40,41). It is defined as the ratio between the area of the surface of the molecule and that of the minimum surface corresponding to the volume of the molecule (that is, the surface of a sphere with a volume that equals the volume of the molecule). Hence, it represents a size and shape descriptor, as it depends on the shape of the molecule and, for common organic molecules, it also scales with size. Our previous 2 structure-activity relationship studies already indicated an important role played by molecular size in this class of soft anticholinergics (9,53). In the present study, molecular size as measured, for example, by volume is still highly correlated with muscarinic binding activity in the analyzed 3 receptor subtypes; however, ovality (O_e) seems to be somewhat more relevant. This suggests that, in addition to size, molecular shape might also influence activity in this series of soft anticholinergics.

For m_1, the best QSAR equation found was $pK_i(m_1) = 0.69 - 5.16(\pm1.23)O_e + 33.57(\pm13.86)q_{C=} - 20.12(\pm6.92)q_{O=}$. This indicates that besides geometric descriptors, electronic properties—namely, the partial atomic charges on the sp^2 carbon ($q_{C=}$) and oxygen ($q_{O=}$) atoms of the ester moiety—also influence the binding affinity of the soft anticholinergic to the m_1 receptor. For m_2, the QSAR equation obtained was $pK_i(m_2) = -3.68 - 3.27(\pm1.19)O_e + 37.57(\pm13.32)\ q_{C=} - 17.84(\pm6.27)\ q_{O=}$. This is similar to that obtained for m_1.

Table 7. Selected physicochemical parameters for soft anticholinergics[a] containing a tropine moiety in their structure.

Compound	*QLogP*	O_e	*D*	*I*	χ	$q_{C=}$	$q_{O=}$
methatropine	−1.47	1.82	13.95	11.79	7.66	0.326	−0.337
methscopolamine	−2.90	1.80	12.24	12.01	7.93	0.323	−0.336
PMTR.Et	−0.52	1.90	18.39	11.95	7.80	0.317	−0.309
PMTR.cHx	1.00	1.99	21.96	11.74	7.69	0.315	−0.303
PMTR.Hx	1.27	2.04	23.04	11.94	7.79	0.316	−0.308
PCMS-1	1.45	1.99	19.65	11.85	7.73	0.319	−0.310
PCMS-2	1.00	1.96	19.21	11.87	7.75	0.319	−0.309
PCPA.Me	1.01	1.99	12.46	11.84	7.82	0.313	−0.335
PCPA.Et	1.46	2.02	11.63	11.82	7.78	0.313	−0.335
PCPB.Me	1.01	1.99	12.60	11.83	7.83	0.313	−0.334
PCPB.Et	1.46	2.03	11.84	11.82	7.79	0.313	−0.335
PCHA.Me	0.83	1.96	12.06	11.29	7.51	0.318	−0.321
PCHA.Et	1.28	1.99	11.30	11.19	7.44	0.317	−0.326
PCHB.Me	0.83	1.96	12.35	11.19	7.47	0.319	−0.320
PCHB.Et	1.28	2.00	11.63	11.18	7.44	0.319	−0.320
TMTR.Et	−0.73	1.88	18.28	11.57	7.61	0.317	−0.308
TMTR.iPr	−0.28	1.92	18.86	11.57	7.60	0.318	−0.310
PMTR.TR	2.52[b]	2.05	11.93	13.76	9.43	0.328	−0.316
PSTR.TR	2.97[b]	2.09	16.51	13.87	9.56	0.295	−0.322
PMTR.MeSOMe	−2.71	1.95	18.58	11.40	7.50	0.318	−0.308

[a] For comparison, the data of methatropine and methscopolamine were also included.
[b] For these structures with 2 tropine moieties, correction for only 1 quaternary nitrogen was included, as there is no definite proof of additivity in such cases with 2 charged groups.

Table 8. Regression equations.

No.	*Equation*	SE	*r*	*F*
1	$pK_i(m_1) = 0.69 - 5.16(\pm1.23)O_e + 33.57(\pm13.86)q_{C=} - 20.12(\pm6.92)\, q_{O=}$	0.33	0.89	19.51
2	$pK_i(m_2) = -3.68 - 3.27(\pm1.19)O_e + 37.57(\pm13.32)\, q_{C=} - 17.84(\pm6.27)\, q_{O=}$	0.32	0.85	14.43
3	$pK_i(m_3) = 20.96 - 6.64\,(\pm1.83)O_e$	0.59	0.65	13.08
4	$pK_i(m_3) = 9.51 - 0.10(\pm0.04)D$	0.65	0.54	7.47
5	$pK_i(m_3) = 8.06 - 0.18\,(\pm0.11)$QLogP	0.71	0.39	3.14
6	$pK_i(m_3) = 22.63 - 6.66(\pm1.32)O_e - 0.10(\pm0.02)D$	0.42	0.85	21.61
7	$pK_i(m_3) = 30.99 - 11.06(\pm2.48)O_e - 0.09(\pm0.02)D + 0.24(\pm0.12)$QLogP	0.39	0.88	18.45

Table 9. Correlation matrix between selected physicochemical descriptors and pK_i values.

	QLogP	O_e	*D*	$q_{C=}$	$q_{O=}$	$pK_i(m_1)$	$pK_i(m_2)$	$pK_i(m_3)$
QLogP	1	0.867	−0.115	−0.431	0.009	−0.582	−0.445	−0.385
O_e	0.867	1	−0.038	−0.534	0.082	−0.792	−0.700	−0.647
D	−0.115	−0.038	1	−0.100	0.776	−0.254	−0.373	−0.526
$q_{C=}$	−0.431	−0.534	−0.1	1	0.091	0.619	0.642	0.494
$q_{O=}$	0.009	0.082	0.776	0.091	1	−0.362	0.383	0.371
$pK_i(m_1)$	−0.582	−0.792	−0.254	0.619	−0.362	1	0.916	0.834
$pK_i(m_2)$	−0.445	−0.700	−0.373	0.642	−0.383	0.916	1	0.873
$pK_i(m_3)$	−0.385	−0.647	−0.526	0.494	−0.371	0.834	0.873	1

For the m_3 receptor binding data, details on the stepwise improvement of the linear regression for the soft anticholinergic agents with a tropine moiety in their structure are included in Table 8. The pK_i (m_3) were correlated in decreasing order with ovality (O_e, r = 0.65), dipole moment (D, r = 0.53), and calculated lipophilicity (QLogP, r = 0.39). Lipophilicity has been long recognized as an important factor determining antimuscarinic activity (54-56). Because O_e and D are essentially perpendicular (show very little intercorrelation: r = –0.038, Table 9) and both of them are well correlated with $pK_i(m_3)$, the combination gives a reasonably good description:

$pK_i(m_3) = 22.63 - 6.66(\pm1.32)O_e - 0.10(\pm0.02)D$

$n = 18$, $r = 0.85$, SE = 0.42, $F = 21.61$

This clearly indicates that for the compounds included here, both size/shape (O_e) and electronic properties (D) influence receptor binding. Addition of a lipophilicity parameter, QLogP, (eq. 7, Table 8) provides some improvement in the correlation (r = 0.88, SE = 0.39) and is statistically justified (at a $p < 0.06$ level), but because on the included data O_e and QLogP are intercorrelated, the improvement is less significant.

DISCUSSION

It has to be mentioned again that unlike in our previous studies on guinea pig ileum pA_2s (9,53), in this study size descriptors do not account for a majority of the variance in activity; in addition, the role of electronic properties seems to be more important. Part of the reason for this deviation might be the fact that pA_2 values are not as closely related to the contraction mediated by the M_3 receptor subtype as previously thought. The latest research indicates that muscarinic receptors in guinea pig ileum are heterogeneous with a major M_2 receptor population (~80%) and a minor M_3 population (~20%). M_3 has been clearly shown to mediate contraction, but the role M_2 plays in smooth muscle contraction is not completely clear at present. Its role might be related to the inhibition of the relaxation of the smooth muscle (33,50). Therefore, pK_i (m_3) values, which are solely related to the m_3 receptor subtype, could deviate from pA_2 values, which might be related to mediation by both M_2 and M_3 receptor subtypes.

The electronic character of the molecules has already been considered as one of the most important parameters related to the antagonist activity of anticholinergic agents (54,57,58). This is particularly true for the present set of molecules. The presence of a free hydroxyl group in methatropine and methscopolamine and the presence of an ester group near the tropine quaternary nitrogen head in several compounds will certainly influence the overall binding ability of the compounds to the muscarinic receptor. In an aqueous environment, the effect of electrostatic interactions may less important, but in a secluded receptor-ligand complex, the electronic effects of various substituents may easily alter the interaction of the ligand with the receptor. Many investigators have noted that esters with an OH group in the acyl side chain were generally associated with greater antimuscarinic activity. Recanatini and

co-workers (59) proposed that the presence of the OH group might influence the conformation of the molecule so that the group might interact with additional binding sites at the receptor. Other investigators related such increases to the change of the electronic properties of the overall molecule caused by the electronic withdrawing properties of the OH group (58) or to its ability to interact by electrostatic interaction or hydrogen bonding with the receptor (57). Our results seem to support the latter suggestions because electronic properties have been shown to be major factors determining antimuscarinic activity in our present QSAR study.

CONCLUSION

In conclusion, the receptor binding assay based on cloned human muscarinic receptors was proved to be a valid screening method to determine the potency of soft anticholinergic agents. Two soft anticholinergics were shown to have moderate muscarinic receptor subtype selectivity. A QSAR study performed on soft anticholinergics containing tropine moiety within their structures showed that the pK_i values are related to geometric properties (O_e), eletronic parameters ($q_{C=}$, $q_{O=}$, and D), and lipophilicity (QLogP).

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